Two photon laser scanning microscopy and true N&B analysis allowed determination of the absolute concentration of GFP molecules inside the bacterial cells. We collected data on hundreds of B. subtilis cells expressing GFP under control of the promoters of interest and grown under glycolytic or gluconeogenic conditions. Results showed no regulation of the promoter expressing the gluconeogenic repressor, strong repression of the gluconeogenic enzyme promoters and weak auto-repression of the glycolytic promoter, with a highly asymmetric distribution when repressed. All promoters showed strong evidence for transcriptional bursting. Analysis of the data using stochastic models of gene expression is currently underway. The figure shows number maps of bacterial cells grown on glucose(G) or Malate(M). Each change in color represents 10 molecules up to 180.

PLATFORM T: DNA, RNA Structure & Conformation/RNA Folding

968-Plat
Flexibilities of Single-Stranded Nucleic Acids Measured by Single Molecule FRET
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The flexibilities of single-stranded RNA has not been as well studied as double-stranded RNA, despite their significant biological importance. Their values for persistence lengths have often been inferred from that of single-stranded DNA. In this poster, we compare the persistence lengths of poly rU and poly-dT as measured by single molecule FRET experiments. We show differences in flexibilities when the nucleic acids are in the presence of monovalent and divalent salts.

969-Plat
Mechanical Unfolding of the Beet Western Yellow Virus −1 Frameshift Signal
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Using unfolding by optical tweezers and steered molecular dynamics (SMD) simulations we have demonstrated the critical importance of Mg^{2+} ions for the mechanical stability of the BWYV RNA pseudoknot. The optical tweezers experiments pointed to a critical role of stem 1 of the pseudoknot, a finding that was confirmed using the SMD simulations. These simulations supported the notion that the stability of stem 1 is critical for −1 frameshifting, a translational recoding event essential for replication of the BWYV. Furthermore, they clarified the precise role of two Mg^{2+} ions, Mg45 and Mg52, in −1 frameshifting. The ions were shown to play a critical role in stabilizing stem 1 by two possible mechanisms depending upon the hydration of the Mg^{2+} ions. Mg^{2+} ions were either directly forming a salt bridge between the strands of stem 1, or they stabilized parallel orientation of the strands in stem 1. Interestingly, these findings explain the drop in frameshifting efficiency, down to null levels, of the C8U mutant. The large effect of this mutant upon the frameshift efficiency seemed surprising as only a single hydrogen bond appeared to have been sacrificed. However, the SMD simulations clarify how the C8U mutation affects the Mg^{2+} coordination and destabilizes stem 1 of the pseudoknot.

970-Plat
Two Distinct Overstretched DNA States
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Double-stranded DNA can undergo an ''overstretching'' transition within a narrow force range when the external force reaches around 60 pN. The basic question of the overstretched DNA is whether the strands are separated or not after transition. Despite numbers of studies, this question still remains controversial. In this research, we directly show that DNA overstretching transition actually involves two distinct types of double-helix reorganization: slow hysteretic ''unpeeling'' of one strand off the other; and a fast, non-hysteretic transition to an elongated double stranded form. The competing between these two overstretched forms is sensitive to factors that affect DNA base pair stability: DNA sequence, salt concentration, and temperature. The balance between the two forms shifts near physiological solution conditions. This result clearly demonstrates that an overstretched double stranded state does exit. It also shows that DNA double helix physical properties, unpeeling or overextension, can be selected via small changes in molecule environment.

971-Plat
Magnetic Torque Tweezers and Their Application in Probing the Torsional Properties of DNA, RNA, and DNA Filaments
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The double-stranded nature of DNA links processes such as replication, transcription, and repair, to rotational motion and torsional strains. Magnetic tweezers (MT) are a powerful single-molecule technique to apply both forces and torques to individual molecules. However, while the forces applied in conventional MT can be calibrated from thermal fluctuations or computed from first principles [1], direct measurement of the torque is challenging.
Here we present the magnetic torque tweezers (MTT), which enable the direct measurement of torque [2] based on a tracking protocol that monitors x, y, z, and angle and on a redesigned magnet configuration. We have applied the MTT to dsDNA, dsRNA, and RecA-DNA heteroduplex filaments. Our measurements of the effective torsional stiffness C of dsDNA indicate a significant force dependence, with $C \approx 40$ nm at low forces up to $C \approx 100$ nm at high forces, reconciling previous partially conflicting measurements. Torque measurements on RecA-DNA heteroduplex filaments reveal an initial torsional stiffness about two-fold higher than that of DNA. However, at relatively moderate torques further build-up of torsional strain is prevented by partial RecA disassembly and structural transitions in the filament. Preliminary results on the torsional properties of fully dsRNA indicate static properties overall similar to dsDNA, but significantly different dynamics of supercoil formation.
Finally, we compare the MTT configuration with recently developed strategies to measure torque using optical tweezers [3] and with a related MT approach that allows straight-forward measurements of free rotation termed freely-orbiting magnetic tweezers [4].
[1] Lipfert, Hao & Dekker, *Biophys. J.* (2009).
[2] Lipfert, Kerssemakers, Jager & Dekker, *Nature Methods, in press* (2010).
[3] Forth, *et al. Phys. Rev. Lett.* (2008).
[4] Lipfert, Wiggin, Kerssemakers & Dekker, *submitted* (2010).

972-Plat
Structural Characterization of Torsional Destabilization in DNA
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Within cells, supercoiled DNA is inherently subjected to various degrees of bending and twisting, and many DNA-binding proteins are now known to be sensitive to these mechanical features of DNA. Under significant levels of bending and/or torsional stress, DNA has been shown to depart from native B-form structure and adopt a number of more energetically favorable alternate conformations. However, very little is known about the structural details of these stress-induced structures. Recently, the DNA nuclease BAL 31 was used to assay for helical destabilizations in small DNA minicircles sustaining fixed amounts of bending and torsional stresses (Nucl. Acids Res., 36(4), 2008). Here, we seek to determine if the helical destabilizations that accompany elevated levels of negative torsional stress in tightly looped minicircles produce localized structures that confer enhanced bending flexibility to the DNA structure, such as would occur in kinked DNA. Towards this end, we synthesized untwisted, 100-bp minicircles that are recognized and digested by BAL 31 and overtwisted 106-bp minicircles that are resistant to degradation by BAL 31. Using cryo-electron microscopy, we then generated three-dimensional image reconstructions of these two minicircle species. From these quantitative descriptions of the minicircle geometries, we observe no evidence of DNA kinking in either the BAL 31-sensitive, 100-bp minicircles or the BAL 31-insensitive, 106-bp minicircles. Since the torsional destabilizations that are recognized by BAL 31 were not observed to confer significant enhancements in bending flexibility, we propose that the torsional destabilizations appearing with the 100-bp minicircle relieve negative torsional stress through the formation of a left-handed dinucleotide stack with no interruption in base-stacking at the right-to-left transition, thus minimally affecting the local bending stiffness. Our observations are consistent with the formation of Z(WC)-DNA, which has been previously theorized to be the predominate form of left-handed DNA appearing in nature.